THE ROYAL SOCIETY

Received 25 July 2003
Accepted 17 November 2003
Published online 2 March 2004

The mode of pheromone evolution: evidence from bark beetles

Matthew R. E. Symonds* **and Mark A. Elgar**

Department of Zoology, University of Melbourne, VIC 3010, Australia

Sex and aggregation pheromones consist of species-specific blends of chemicals. The way in which different species' blends have evolved has been the subject of some debate. Theoretical predictions suggest that differences between species have arisen not through the accruing of small changes, but through major shifts in chemical composition. Using data on the aggregation pheromones of 34 species of bark beetle from two genera, *Dendroctonus* and *Ips*, we investigated how the distributions of the chemical components of their pheromone blends mirror their phylogenetic relationships. We tested whether there were consistent patterns that could be used to help elucidate the mode of pheromone evolution. Although there were obvious differences in pheromone blends between the two genera, the differences between species within each genus followed a less clear phylogenetic pattern. In both genera, closely related species are just as different as more distantly related species. Within *Dendroctonus*, particularly, most chemical components were distributed randomly across the phylogeny. Indeed, for some chemicals, closely related species may actually be more different than would be expected from a random distribution of chemical components. This argues strongly against the idea of minor shifts in pheromone evolution. Instead, we suggest that, within certain phylogenetic constraints, pheromone evolution in bark beetles is characterized by large saltational shifts, resulting in sibling species being substantially phenotypically (i.e. pheromonally) different from one another, thus agreeing with theoretical predictions.

Keywords: Scolytidae; Coleoptera; aggregation pheromones; phylogeny; computer simulation; saltational evolution

1. INTRODUCTION

Many organisms use pheromones as signals to attract mates for sex or other conspecifics for resource exploitation. These pheromones consist of particular blends of chemicals that are typically species specific (Roelofs 1995). The result is not only an extraordinary diversity of pheromone blends, but also a remarkable convergence of particular blends across different taxa. While many studies of chemical communication now incorporate evolutionary approaches (Löfstedt 1993; Vet 1999; Ayasse *et al.* 2001; Wyatt 2003), the evolutionary processes that have generated the diversity of pheromone blends remain poorly understood.

Traditionally, pheromone blends were thought to have evolved in a characteristically Darwinian fashion through gradual changes in the proportions and structures of the chemicals involved over time (e.g. Roelofs & Brown 1982). However, the high species specificity of pheromones suggests that there should be strong selection against small modifications in these signals, and thus gradual evolution of pheromones through small changes in chemical components is unlikely (Paterson 1985). Instead, it seems more likely that pheromone evolution occurs via sudden major shifts ('saltational shifts' following Baker (2002)) in the pheromone constituents (Löfstedt 1993). Butlin & Trickett (1997), using computer simulation, offer some support for this view. More recently, Roelofs *et al.* (2002) provided the first empirical corroboration by showing that substantial shifts in the sex-pheromone blends of two corn borer moth species (genus *Ostrinia*) could be obtained by the activation of a single gene transcript (the Δ14-desaturase gene). As these authors pointed out, the implications for the control of pest species with this evolutionary ability suddenly to shift blends are immense. Chemical means of control (such as using pheromone mimics to trap individuals or inhibit their response to their own species' pheromones) may ultimately prove as difficult as is the control of certain bacteria with antibiotics, and hence we may see pest species acquiring 'pheromone resistance' in the future. While the work of Roelofs *et al.* (2002) is a significant advance in our understanding of pheromone evolution, their study was restricted to a single pair of species. Further investigations of a wider range of organisms are therefore warranted.

The chemical ecology of bark beetles (Scolytidae: Coleoptera) has been well studied (Byers 1989; Seybold *et al.* 2000; Raffa 2001), primarily because some species attack live trees and hence are commercially important pest species (Wood, D. L. 1982). Many bark beetles produce aggregation pheromones that act as attractants to conspecifics, causing them to gather together in considerable numbers to mate and/or feed communally (see Borden (1985) for a review). In addition, a large number of species produce specific repellent or anti-aggregation pheromones or pheromones that are multifunctional (i.e. are attractive at low concentrations but repellent at high concentrations) because aggregation may ultimately lead to excessive intraspecific competition for mates or food resources (Raffa 2001).

There is considerable diversity among the bark beetles in the blends of chemicals that constitute their aggregation

*Author and address for correspondence: School of Tropical Biology, James Cook University, Townsville, QLD 4811, Australia (matthew.symonds@jcu.edu.au).

Table 1. Groupings of the 25 pheromone components used in the analyses based on structural similarities.

group 1	frontalin
	exo-brevicomin
	endo-brevicomin
group 2	*cis*-verbenol
	trans-verbenol
	verbenone
	verbenene
	myrtenol
	pinocarvone
group 3	heptanol
group 4	seudenol
	1-methyl-2-cyclohexen-1-ol (MCOL)
	3-methyl-2-cyclohexenone (3,2-MCH)
group 5	1-phenylethanol
	2-phenylethanol
group 6	ipsenol
	ipsdienol
	amitinol
	E-myrcenol
	lanierone
group 7	2-methyl-3-buten-2-ol
	3-methyl-3-buten-1-ol
	3-methyl-3-buten-2-ol
	trans-pentenol
group 8	3-caren-10-ol

pheromones (Borden 1985; Seybold *et al.* 2000). This prompts the questions of how and why this diversity arose. Previous studies of bark beetles have produced contradictory results. Cane *et al.* (1990) suggested, in a study of seven species of *Ips*, that the pheromone-communication system of these beetles paralleled their evolutionary relationships. Francke *et al.* (1995), however, expressed the view that there was little correlation between the pheromone chemistry and phylogeny of bark beetles. In a subsequent more rigorous analysis of 10 *Ips* species, Cognato *et al.* (1997) found a fairly high degree of homoplasy (i.e. convergent or parallel evolution) within the pheromone components, indicating that pheromone evolution is plastic and not congruent with phylogeny. This result is consistent with the idea of major shifts in pheromone evolution.

These studies, however, have two major limitations. First, their small taxonomic scale prevents them from investigating the mode of pheromone evolution in detail. Second, their focus on the *production* of particular chemicals, rather than on whether these chemicals actually function as species-specific signals, makes it more difficult to apply their results to the evolution of communication systems.

We analysed the aggregation-pheromone blends of species from two genera of bark beetle, *Dendroctonus* and *Ips*. We examined whether the mode of pheromone evolution in this family fits the predictions from models of minor or major changes by mapping chemical components known to be functionally active among these species onto their evolutionary tree. Under a model of gradual minor change, closely related species should have a strong similarity in their pheromone blends, with more distantly related species showing increasing phenotypic difference. Alternatively, a model consisting of saltational shifts should produce a considerable difference in blends between closely related species, with more distantly related species showing far less, or perhaps no, increase in the levels of pheromonal difference. We also expand the analysis of Cognato *et al.* (1997) to include more species of *Ips* and, for the first time, members of the genus *Dendroctonus*. This provides a more detailed picture of the relationship between pheromone diversity and phylogeny, both within and between genera.

2. METHODS

We collated data from the literature on 25 different chemical constituents ('characters') of the aggregation pheromones for 34 species of bark beetle from the genera *Dendroctonus, Ips* and *Orthotomicus* (which is very closely related to and may be synonymous with *Ips*; Wood, S. L. 1982). For each species we scored a chemical as present if it was both produced by the species and recorded as an active component of its aggregation-pheromone blend. Note that differences between our dataset and that of Cognato *et al.* (1997) result from the distinction between 'produced' (Cognato *et al.* 1997) and 'actively functional' (this study) pheromone components. We also counted the chemical as present if it was multifunctional or actively repellent. As with any literature-based comparative analysis, the results are subject to the quality of the data that are available. Some unevenness in the data is inevitable because some species and chemicals have been more intensively studied than others, and because researchers have employed different experimental and analytical methods. However, we cannot identify any way in which this data unevenness should cause a systematic bias towards type I or type II errors in our analyses.

The chemical characters were then mapped onto recently published molecular-based phylogenies of *Ips* (including *Orthotomicus*) (Cognato & Sperling 2000, fig. 5) and *Dendroctonus* (Kelley & Farrell 1998, fig. 2*a*). The combined phylogeny, plus character states, is shown in figure 1. A list of the sources for the pheromone data is presented in electronic Appendix A.

(a) *Congruence with phylogeny*

We assessed the degree to which the pheromone components were congruent with phylogeny by calculating three measures: (i) the number of chemical-character changes along the phylogeny (i.e. the number of steps, s), (ii) the degree of homoplasy (the homoplasy index (HI) as used by Cognato *et al.* (1997), $\mathrm{HI} = 1 - m/s$, where m is the minimum possible number of steps), and (iii) the degree of phylogenetic independence (PI) (as developed by Björklund (1997), $\mathrm{PI} = 1 - (g - s)/(g - m)$, where g is the maximum possible number of steps for a given phylogeny). For this part of the analysis we did not include phylogenetically uninformative chemical components that were unique to a particular species (e.g. lanierone for *I. pini*; Teale *et al.* 1991). The three parameters were calculated for the two genera separately, in each case using only those characters shared by two or more of the species in the genus. The character dataset was then randomly shuffled holding the phylogeny constant (using the shuffle option in MacClade 4; Maddison & Maddison 2001), and the values for the three measures were calculated using this randomly perturbed dataset. This procedure was repeated 500 times to generate a random distribution for each of the three measures (see Björklund 1997), which was then

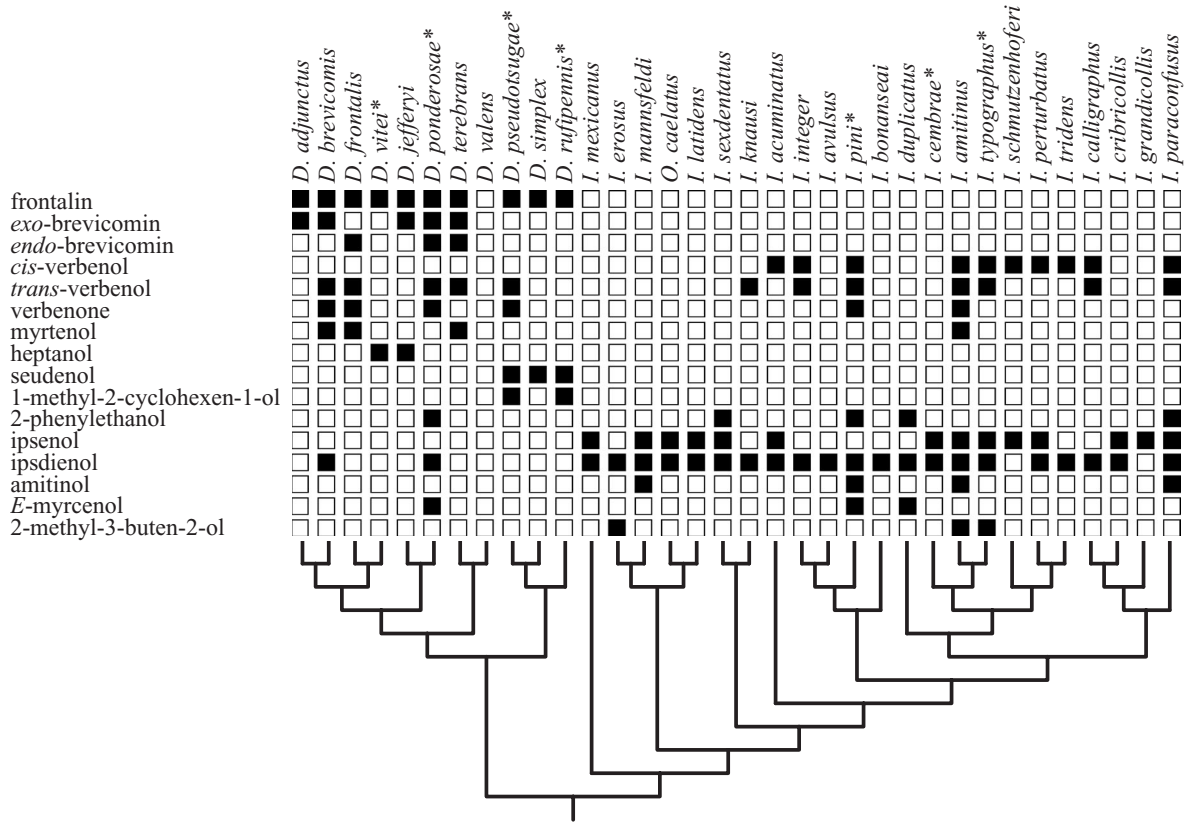

Figure 1. Phylogeny of *Dendroctonus* and *Ips*/*Orthotomicus* species used in the analysis, with pheromone components superimposed. Filled squares indicate the presence of a chemical component as part of the active pheromone blend for a species. An asterisk indicates species that have pheromone components that are unique to them (not shown on the figure). These are: *D. ponderosae,* pinocarvone and 3-caren-10-ol; *D. pseudotsugae,* 3-methyl-2-cyclohexenone (3,2-MCH) and *trans*-pentenol; *D. rufipennis,* verbenene; *D. vitei,* 1-phenylethanol; *I. cembrae,* 3-methyl-3-buten-1-ol; *I. pini,* lanierone; and *I. typographus,* 3-methyl-3-buten-2-ol.

compared with the real data to test whether the phylogenetic distribution of pheromone components was more conservative than would be expected by chance.

(**b**) *Mode of pheromone evolution*

We then tested how differences in pheromone blends were related to phylogeny. For each and every pair of species we counted the number of phenotypic differences in the pheromone blends (i.e. the number of chemicals that were absent in one species but present in the other). We obtained a rough measure of the phylogenetic distance between each pair of species by counting the number of nodes in the phylogeny separating the two (following Hansen & Martins 1996). Sibling species were therefore assigned a phylogenetic distance of one, and so on. To produce distances more representative of the true phylogeny and number of speciation events, we calculated these distances using the phylogeny of all the species that were used in the source trees (i.e. including species for which we had no chemical data). The mean amount of phenotypic difference was then calculated for each level of phylogenetic distance. Results were generated for each genus (*Dendroctonus*, *Ips–Orthotomicus*) separately, and a value for the mean difference between the two genera was also calculated.

The biosynthetic pathways of many bark beetle semiochemicals are still uncertain. The chemicals may be produced by sequestration and modification of host-tree compounds, through microbially assisted synthesis by symbiotic bacteria or fungi, or by separate pathways whereby they are manufactured *de novo* by the beetles themselves (see review in Seybold *et al.* (2000)). However, close structural similarities suggest that certain chemicals may share common synthetic pathways. Therefore, phenotypic differences between some chemical blends might not be as profound as those between blends consisting of structurally dissimilar chemicals. Hence some of the characters in our analysis may not be truly independent of each other. We accounted for this possibility by repeating the above analysis using groups of chemical structures as phenotypic characters. These groupings are listed in table 1.

We compared the pattern in our results with those obtained from 100 computer-simulated datasets of eight hypothetical characters for our 34 species. These were generated using the known complete phylogeny and the 'evolve characters' option in MacClade. Each character was assigned a random ancestral state (i.e. 0 or 1 with equal probability) and could then change to the other state along a branch according to an assigned probability (see below). Change was allowed in only one branch following each bifurcation in the phylogeny. The probability of change was assigned according to one of three simulation models.

(i) A 'minor change' model, where the mean difference between two sibling species in the phylogeny was one character in eight. The probability of change in a single character state following a bifurcation was therefore 0.125.

Table 2. Measures of congruence with phylogeny of the pheromone-component data for the two bark beetle genera, comparing the real data with the expected mean result from randomly perturbed data on the same phylogeny.
(The statistical significances of any differences were assessed using two-tailed *Z*-tests. (*a*) Individual pheromone-component data; (*b*) Grouped pheromone-component data.)

genus	dataset	number of steps	HI	PI
(*a*)				
Dendroctonus	pheromone	25	0.64	0.80
	perturbed (± s.d.)	25.22 ± 1.94	0.64 ± 0.04	0.81 ± 0.09
	Z	−0.12	−0.01	−0.13
	p	0.45	0.50	0.45
Ips	pheromone	35	0.74	0.76
	perturbed (± s.d.)	37.71 ± 2.05	0.76 ± 0.01	0.84 ± 0.06
	Z	−1.32	−1.58	−1.36
	p	0.09	0.06	0.09
(*b*)				
Dendroctonus	pheromone group	11	0.55	0.75
	perturbed (± s.d.)	11.44 ± 1.04	0.56 ± 0.04	0.80 ± 0.13
	Z	−0.42	−0.22	−0.40
	p	0.34	0.41	0.34
Ips	pheromone group	13	0.77	0.71
	perturbed (± s.d.)	13.71 ± 1.29	0.78 ± 0.02	0.77 ± 0.09
	Z	−0.55	−0.48	−0.58
	p	0.29	0.32	0.28

(ii) An 'intermediate change' model, where the mean difference between two sibling species was three characters in eight (i.e. 0.375 probability of change per character).
(iii) A 'major change' model, where the mean difference between two sibling species was six characters in eight (i.e. 0.75 probability of change per character).

Note that in the last case the degree of difference (six characters) between sibling species will be greater than that expected by chance alone (four characters).

3. RESULTS

(a) *Congruence with phylogeny*

The degree of congruence of the data with the phylogeny, comparing the chemical data with the randomly perturbed data, is shown in table 2. All three measures (i.e. number of steps, HI and PI) indicate that the phylogenetic distribution of the nine informative pheromone components among the species of *Dendroctonus* is not significantly different from that expected by chance. In other words, the pheromone components are essentially randomly distributed across taxa. Nevertheless, it is obvious that some of the chemicals *are* restricted to certain clades within the genus. Most notably, seudenol and 1-methyl-2-cyclohexen-1-ol (MCOL) are found exclusively in the clade consisting of *D. pseudotsugae*, *D. simplex* and *D. rufipennis*. Likewise, the absence of *exo*-brevicomin and *endo*-brevicomin from these species' blends hints at a phylogenetic pattern. However, the phylogenetic distributions of *trans*-verbenol, verbenone and myrtenol are such that closely related species in the phylogeny are more different from each other than would be expected by chance (considering just these three characters: observed number of steps = 13, expected = 11.33 ± 0.42, $Z = 3.76$, $p < 0.001$).

Within the genus *Ips*, the eight informative characters show more congruence with phylogeny. In particular, the number of character steps is fewer and the HI and the degree of PI of the data are smaller than would be expected by chance. Even so, this pattern is fairly weak (see table 2). Some of the chemicals do appear to be randomly scattered among the species (e.g. 2-phenylethanol and amitinol), but others fit the phylogeny more closely. For example, *cis*-verbenol is found exclusively in the clade containing *I. acuminatus* and *I. paraconfusus*, and ipsenol is absent in the four-species clade containing *I. pini*.

No phylogenetic pattern to the data emerges when considering the pheromone groups as individual characters. These results argue against the idea that closely related species become phenotypically different in their pheromone blends simply by switching to a different set of closely related chemicals.

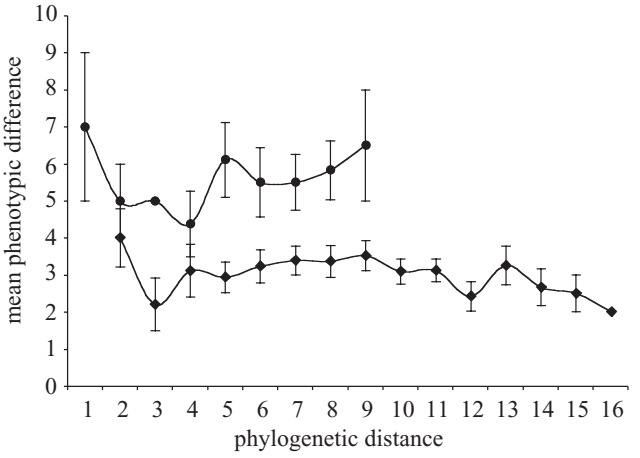

Figure 2. Mean (± s.e.) phenotypic differences in pheromone-blend components between species plotted against their phylogenetic distance from each other using data for the two bark beetle genera used in the analysis (circles, *Dendroctonus*; diamonds, *Ips*).

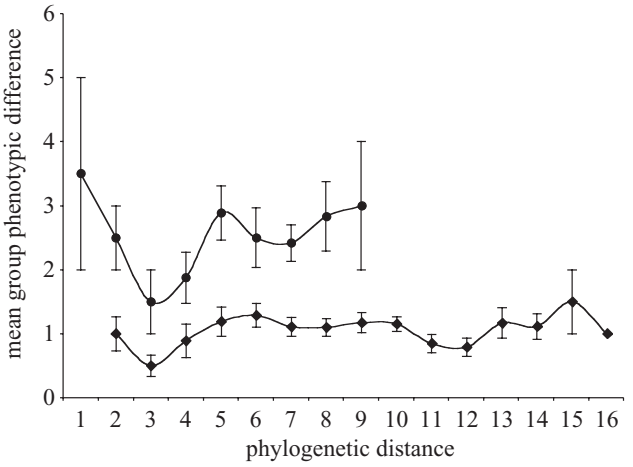

Figure 3. Relationship between mean (± s.e.) phenotypic difference in structural groups of pheromone components and phylogenetic distance between species for the two bark beetle genera used in the analysis (circles, *Dendroctonus*; diamonds, *Ips*).

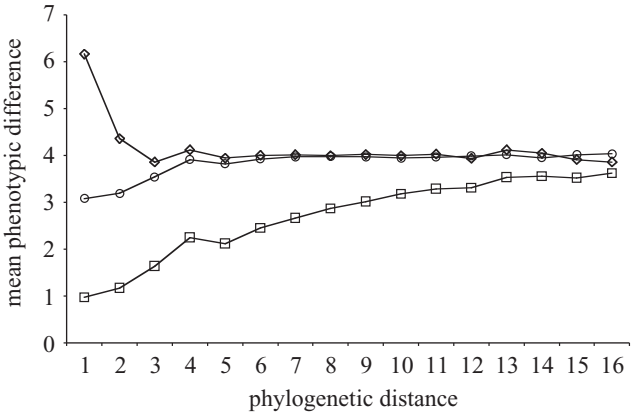

Figure 4. Predicted relationship between phenotypic difference and phylogenetic distance in bark beetles for eight hypothetical characters simulated under three different models of evolution (see § 2b for details). Squares, 'minor change' model; circles, 'intermediate change' model; diamonds, 'major change' model.

(b) *Mode of pheromone evolution*

Figure 2 illustrates how the mean amount of phenotypic difference between species in each genus is related to their phylogenetic distance. Generally speaking, there is neither an increase nor a decrease in phenotypic difference with increasing relatedness. Closely related species are just as different as more distantly related species. There are obvious differences between the two genera, with *Dendroctonus* species alone using frontalin, brevicomin, heptanol, seudenol, MCOL and pinocarvone, and *Ips* species alone using *cis*-verbenol, ipsenol, amitinol and 2-methyl-3-buten-2-ol. The mean phenotypic difference between the two genera is 6.613 ± 0.156 (mean ± s.e.) chemicals. While this is two to three times greater than any differences observed within *Ips*, it is not much greater than the differences observed within *Dendroctonus*. Indeed, for the two pairs of sibling species in this genus for which we have data (*D. jeffreyi–D. ponderosae* and *D. pseudotsugae–D. simplex*), the phenotypic difference (7 ± 2.00) may be even greater than the mean difference between *Dendroctonus* and *Ips* species. Similarly, there is apparently no change in phenotypic difference in the groups of pheromones used as the species become more phylogenetically distant, at least within each genus (see figure 3). The two genera may be substantially different (mean difference = 3.273 ± 0.072), but again sibling species in *Dendroctonus* show even greater differences (mean = 3.5 ± 1.5).

Likewise, the *Dendroctonus* data suggest that sibling species may be more phenotypically different than are other species within the same genus, although we reiterate that this result is based on only two comparisons. Unfortunately, when the full phylogeny of *Ips* is taken into account, no sibling-species comparisons are possible using our data.

Figure 4 shows the patterns of the relationship between phylogenetic distance and phenotypic difference predicted using the data generated by the three different evolutionary models. The bark beetle results clearly show a lack of fit with the 'minor change' model, where there is a steady increase in phenotypic difference with increasing phylogenetic distance. The 'intermediate change' model also shows an increase in phenotypic difference as the species become more phylogenetically separated, albeit at a slower rate. However, it is apparent that phenotypic difference increases towards an asymptote of around four, the mean amount of difference expected between two species for eight randomly assigned dichotomous characters. At this level of difference, any random changes in character states following a bifurcation are just as likely to make the species more phenotypically similar as more different. The 'major change' model, where sibling species are forced to be more different than would be expected at random, shows a fairly rapid decrease in phenotypic difference to the asymptotic mean level of difference of four as phylogenetic distance increases. These latter two models fit the bark beetle data better, although the lack of any increase in phenotypic difference and the suggestion (at least for *Dendroctonus*) of a decrease hint at stronger support for the 'major change' model.

4. DISCUSSION

(a) *Pheromones and phylogeny*

The way in which bark beetle pheromone components are distributed on the phylogeny differs between *Dendroctonus* and *Ips*. In general there is little congruence between the distribution of the pheromone components and the phylogeny for *Dendroctonus*. However, this disguises the fact that some pheromone components are phylogenetically constrained. As we described in § 3a, the pheromone blends of the clade containing *D. pseudotsugae, D. simplex* and *D. rufipennis* have particular characteristics not found in those of other species of *Dendroctonus*. These three species specialize on the tree genera *Pseudotsuga* (Douglas-fir), *Picea* (spruce) and *Larix* (larch), while the genus *Pinus* (pine) is the primary host for other species of *Dendroctonus* (Kelley & Farrell 1998). This suggests that the presence of particular characteristic chemicals may be associated with the diet of these three species. A link between diet and pheromones has been suggested by other work on bark beetles (e.g. Renwick *et al.* 1976). By contrast, other pheromone components such as *trans*-verbenol, verbenone and myrtenol are more scattered in this genus

than would be expected by chance, suggesting that in some aspects closely related species may actually have evolved to be phenotypically different from each other.

By comparison, the distribution of chemical characters in *Ips* is more congruent with phylogeny, which argues against Cognato *et al.*'s (1997) conclusion that pheromone evolution in *Ips* is plastic and not strongly related to phylogeny. However, like them, we do find that certain chemicals are more phylogenetically independent than others. Clearly, any conclusion depends on the species used and the chemical data employed, with our larger dataset perhaps providing a more comprehensive picture, while the results obtained by Cognato *et al.* indicate that there is less of a phylogenetic pattern within certain clades in *Ips*.

There are obvious differences in the chemicals used in the pheromone blends between the genera. As many *Ips* and *Dendroctonus* species share the same host trees and geographical range (Raffa 2001), it is unlikely that these genus-level differences are the result of different diets or environments. It is possible that the semiochemicals are associated with the mating system and colonization behaviour of the species. In the primarily monogamous genus *Dendroctonus,* only females initiate the colonization of a new tree and produce the aggregation pheromone, whereas in the mainly polygamous genus *Ips,* the males are responsible (Wyatt 2003). Cognato *et al.* (1997) found differences between the sexes in the production of chemicals for *Ips,* although the sexes have different behavioural roles. As we have only one evolutionary comparison between male colonizers (*Ips*) and female colonizers (*Dendroctonus*), we are unable to draw any conclusions about the role of sex differences in the evolution of aggregation pheromones.

It is also interesting to note, when using pheromone components as evolutionary characters, that the most parsimonious tree topologies are considerably shorter than those used in our analysis (18 and 25 steps for *Dendroctonus* and *Ips*, respectively, compared with our data values of 25 and 33 steps). Assuming that the molecular-based phylogenies are reliable, our results reveal a danger in using pheromones as characters for phylogenetic reconstruction. This may be especially so if, as seems likely for *Dendroctonus*, sibling species are more different from each other in certain chemicals than would be expected by chance.

(**b**) *The mode of pheromone evolution*

We find no evidence that bark beetle aggregation-pheromone blends evolve by gradual changes. Species do not slowly accrue small changes in their blends as they diverge from each other, nor do they merely alter the relative proportions of the chemicals in their blends. This result is intriguing because there is considerable evidence from bark beetles of within-species inter-population variation in blend proportions of pheromones. For example, different North American populations of *I. pini* produce and respond to different proportions of the semiochemicals ipsdienol and lanierone in their blends (Seybold *et al.* 1995). Raffa (2001) suggested that this within-species variation may make it difficult for predators to 'tune in' to a signal. In terms of between-species variation, Roelofs & Brown (1982) suggested that the sex pheromones of tortricid moths had evolved by the smallest number of changes in biosynthetic processes at each step. The first stage in this evolutionary process was a change in the relative proportions of chemicals in a blend, followed by switches to structurally related chemicals. Our pheromone-group data do not support this model. Although some phylogenetic patterns in the chemicals are apparent (see § 3a), closely related species do not just produce 'variations on a theme' using the same group of chemicals, but use different groups of chemicals in their pheromones.

The beetle data show closer resemblance to models with higher degrees of phenotypic change at each speciation event. This arises because these models reach an asymptotic mean level of difference fairly quickly, beyond which the amount of phenotypic difference remains constant regardless of phylogenetic distance. Our data seem to show this constant pattern, suggesting that this asymptotic level of difference has been reached, even between sibling species. In contrast to the models, the mean bark beetle level of difference never approaches the value of half the number of chemical characters (i.e. $25/2 = 12.5$) that the simulations suggest. This is because the models assume that each character is free to evolve in either direction (present to absent or absent to present), with the same likelihood, in any branch of the phylogeny (i.e. no character is phylogenetically constrained). However, not all components of bark beetle aggregation pheromones are free from these kinds of constraints. Under such circumstances, the asymptotic mean level of difference will be less than half the number of characters because change in some characters has been constrained to certain lineages (e.g. seudenol and ipsenol), or because change is less likely in some characters (e.g. *E*-myrcenol, heptanol). Additionally, some chemicals (e.g. verbenene, lanierone) will have appeared or become functionally active only after some species had diverged. Clearly, not all bark beetles have the same palette of chemicals from which to work. Nevertheless, the lack of increase in phenotypic difference may reflect the fact that, within certain phylogenetic constraints, bark beetle aggregation-pheromone blends may become as different from each other as would be expected by chance at every speciation event. In other words, each species' blend is a new roll of the particular set of dice they already have.

Our results, for *Dendroctonus* at least, suggest that sibling species may be *more* different from each other than would be expected even by chance. The trend is weak and, we caution, the standard errors are large, but within *Dendroctonus* the highest levels of phenotypic difference are between sibling species (phylogenetic distance of one). In other words, there may be additional selective pressures at work during speciation events that force one pheromone blend to become substantially different from the other. This fits most closely with our 'major change' simulation model. Within *Ips*, this pattern is less clear, although the individual-pheromone data do show that the largest phenotypic difference is at the level of the smallest phylogenetic distance (two). Cognato *et al.* (1997) presented data on chemicals produced by two species, *I. confusus* and *I. hoppingi,* that are very closely related to *I. paraconfusus*, but were not included in this study owing to a lack of functional information (see § 2) and taxonomic concerns.

These data in fact suggest some pheromonal similarity between the three species, which may indicate a more gradual mode of evolution in *Ips*, and more congruence of the pheromone components with phylogeny (contrary to the findings of Cognato *et al.* (1997)). For *Dendroctonus* at least, the data argue that bark beetle aggregation pheromones evolve through sudden saltational shifts at speciation events.

This conclusion raises the question of how this mechanism of evolution actually works. Roelofs *et al.* (2002) have already discovered a genetic mechanism by which large shifts in the chemicals produced as sex pheromones by female cornborer moths can arise. But, to function as a signal, the chemicals must be detected and responded to by appropriate receivers. Roelofs *et al.* (2002) found rare males in the population that were able to respond to these new blends. Species of bark beetle often produce certain chemical components in their blends without responding to them (e.g. verbenone by *D. terebrans*; Phillips *et al.* 1989). Clearly, the evolution of aggregation pheromones as signals depends also on the evolution of the receptors of the receivers. Other factors may also influence the evolution of aggregation pheromones. Most obvious of these is the effect of species overlap. For example, sympatric species may be more phenotypically dissimilar than allopatric species as a result of reinforcement for species isolation (Coyne & Orr 1997). Our conclusions also depend on the phylogeny that we employed. There is evidence that the choice of phylogeny can have profound effects on the conclusions drawn from comparative studies (e.g. Symonds & Elgar 2002).

One final potentially important factor that we do not consider here is that many of the compounds in our analysis are chiral and have two enantiomers. Such optical isomers act essentially as separate compounds, and could be considered as such in this analysis. We did not include chiral information here because of issues of intraspecific variation (e.g. in *I. pini* different populations produce different enantiomeric compositions of ipsdienol; Borden 1985; Cognato *et al.* 1999) and data reliability (not all researchers recorded information on the enantiomer produced). However, if such information were included, the degree of diversity in the chemical characters would be increased. In this situation, it therefore seems likely that the degree of phenotypic difference between species and the incongruence with phylogeny of the pheromone components would be even higher, thereby strengthening our conclusions.

Our study provides, to our knowledge, the first phylogenetic-based comparative support for saltational changes in the evolution of aggregation pheromones. It therefore raises the question of how these changes come about. Following the breakthroughs made by the recent work of Roelofs *et al.* (2002), we suggest that an exploration of the genetic basis of bark beetle aggregation-pheromone production and reception would be highly rewarding.

We thank Deborah Cole for her initial efforts in compiling the data, Tristram Wyatt for early discussions that led to this work, and Robert Capon for discussion of pheromone chemistry. Therésa Jones, and Michael Magrath's comments on the manuscript helped greatly, and we also thank other members of the Animal Behaviour and Evolution Group at Melbourne for their suggestions. M.R.E.S. was supported by a Travelling Fellowship from The Royal Society.

REFERENCES

Ayasse, M., Paxton, R. J. & Tengo, J. 2001 Mating behavior and chemical communication in the order Hymenoptera. *A. Rev. Entomol.* **46**, 31–78.

Baker, T. C. 2002 Mechanism for saltational shifts in pheromone communication systems. *Proc. Natl Acad. Sci. USA* **99**, 13 368–13 370.

Björklund, M. 1997 Are 'comparative methods' always necessary? *Oikos* **80**, 607–612.

Borden, J. H. 1985 Aggregation pheromones. In *Comprehensive insect physiology, biochemistry and pharmacology*, vol. 9 (ed. G. A. Kerkut & L. I. Gilbert), pp. 257–285. Oxford: Pergamon Press.

Butlin, R. K. & Trickett, A. J. 1997 Can population genetic simulations help to interpret pheromone evolution? In *Insect pheromone research* (ed. R. T. Cardé & A. K. Minks), pp. 548–562. New York: Chapman & Hall.

Byers, J. A. 1989 Chemical ecology of bark beetles. *Experientia* **45**, 271–283.

Cane, J. H., Stock, M. W., Wood, D. L. & Gast, S. J. 1990 Phylogenetic relationship of *Ips* bark beetles (Coleoptera: Scolytidae): electrophoretic and morphometric analyses of the *grandicollis* group. *Biochem. Syst. Ecol.* **18**, 359–368.

Cognato, A. I. & Sperling, F. A. H. 2000 Phylogeny of *Ips* DeGeer species (Coleoptera: Scolytidae) inferred from mitochondrial cytochrome oxidase I DNA sequence. *Mol. Phyl. Evol.* **14**, 445–460.

Cognato, A. I., Seybold, S. J., Wood, D. L. & Teale, S. A. 1997 A cladistic analysis of pheromone evolution in *Ips* bark beetles (Coleoptera: Scolytidae). *Evolution* **51**, 313–318.

Cognato, A. I., Seybold, S. J. & Sperling, F. A. H. 1999 Incomplete barriers to mitochondrial gene flow between pheromone races of the North American pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae). *Proc. R. Soc. Lond.* B **266**, 1843–1850. (DOI 10.1098/rspb.1999.0855.)

Coyne, J. A. & Orr, H. A. 1997 'Patterns of speciation in *Drosophila*' revisited. *Evolution* **51**, 295–303.

Francke, W., Bartels, J., Meyer, H., Schröder, F., Kohnle, U., Baader, E. & Vité, J. P. 1995 Semiochemicals from bark beetles: new results, remarks and reflections. *J. Chem. Ecol.* **21**, 1043–1063.

Hansen, T. F. & Martins, E. P. 1996 Translating between microevolutionary process and macroevolutionary patterns: the correlation structure of interspecific data. *Evolution* **50**, 1404–1417.

Kelley, S. T. & Farrell, B. D. 1998 Is specialization a dead end? The phylogeny of host use in *Dendroctonus* bark beetles (Scolytidae). *Evolution* **52**, 1731–1743.

Löfstedt, C. 1993 Moth pheromone genetics and evolution. *Phil. Trans. R. Soc. Lond.* B **340**, 167–177.

Maddison, D. R. & Maddison, W. P. 2001 *MacClade 4: analysis of phylogeny and character evolution. v. 4.03*. Sunderland, MA: Sinauer.

Paterson, H. E. H. 1985 The recognition concept of species. In *Species and speciation* (ed. E. S. Vrba), Transvaal Museum Monograph no. 4, pp. 21–29. Pretoria, South Africa: Transvaal Museum.

Phillips, T. W., Nation, J. L., Wilkinson, R. C. & Foltz, J. L. 1989 Secondary attraction and field activity of beetle-produced volatiles in *Dendroctonus terebrans*. *J. Chem. Ecol.* **15**, 1513–1533.

Raffa, K. F. 2001 Mixed messages across multiple trophic levels: the ecology of bark beetle chemical communication systems. *Chemoecology* **11**, 49–65.

Renwick, J. A. A., Hughes, P. R. & Krull, I. S. 1976 Selective production of *cis*- and *trans*-verbenol from (−) and (+)-pinene by a bark beetle. *Science* **191**, 199–201.

Roelofs, W. L. 1995 The chemistry of sex attraction. In *Chemical ecology: the chemistry of biotic interaction* (ed. T. Eisner & J. Meinwald), pp. 103–117. Washington, DC: National Academy Press.

Roelofs, W. L. & Brown, R. L. 1982 Pheromones and evolutionary relationships of Tortricidae. *A. Rev. Ecol. Syst.* **13**, 395–422.

Roelofs, W. L., Liu, W., Hao, G., Jiao, H., Rooney, A. P. & Linn Jr, C. E. 2002 Evolution of moth sex pheromones via ancestral genes. *Proc. Natl Acad. Sci. USA* **99**, 13 621–13 626.

Seybold, S. J., Ohtsuka, T., Wood, D. L. & Kubo, I. 1995 Enantiomeric composition of ipsdienol: a chemotaxonomic character for North American populations of *Ips* spp. in the *pini* subgeneric group (Coleoptera: Scolytidae). *J. Chem. Ecol.* **21**, 995–1016.

Seybold, S. J., Bohlmann, J. & Raffa, K. F. 2000 Biosynthesis of coniferophagous bark beetle pheromones and conifer isoprenoids: evolutionary perspective and synthesis. *Can. Entomol.* **132**, 697–753.

Symonds, M. R. E. & Elgar, M. A. 2002 Phylogeny affects estimation of metabolic scaling in mammals. *Evolution* **56**, 2330–2333.

Teale, S. A., Webster, F. X., Zhang, A. & Lanier, G. N. 1991 Lanierone: a new pheromone component from *Ips pini* (Coleoptera: Scolytidae) in New York. *J. Chem. Ecol.* **17**, 1159–1176.

Vet, L. E. M. 1999 From chemical to population ecology: infochemical use in an evolutionary context. *J. Chem. Ecol.* **25**, 31–49.

Wood, D. L. 1982 The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. *A. Rev. Entomol.* **27**, 411–446.

Wood, S. L. 1982 The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. *Great Basin Nat. Mem.* **6**, 1–1359.

Wyatt, T. D. 2003 *Pheromones and animal behaviour: communication by smell and taste*. Cambridge University Press.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.

Visit www.journals.royalsoc.ac.uk and navigate to this article through *Proceedings*: Biological Sciences to see the accompanying electronic appendix.